Essential role of GTP in epinephrine stimulation of human fat cell adenylate cyclase

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Abstract The activity of epinephrine-sensitive adenylate cyclase of human fat cell ghosts is markedly enhanced by the GTP analog 5′-guanylyl-imidodiphosphate (GMP-P(NH)P), but a similar effect of GTP itself has not been heretofore demonstrable. In the present work, comparison of adenylate cyclase activity in the presence of epinephrine alone versus epinephrine plus GTP showed that at 37°C GTP doubled activity (10-min incubation); at 30°C less than half this effect was apparent. However, time course studies at both 30 and 37°C showed that comparisons at a single point in time based on ratios of hormone-stimulated activity to basal or basal plus GTP were misleading, since basal activities were not linear with time and were inhibited by GTP. The inhibitory effect of GTP on basal activity was seen at both temperatures but at 37°C decreased with time so that by 10 min the inhibition was no longer apparent. The time course data showed clearly that epinephrine alone did not stimulate adenylate cyclase activity; rather, the hormone merely prevented fall-off of initial rate of unstimulated (basal) enzyme activity. Only when GTP was added together with epinephrine was an unequivocal stimulation of enzyme activity observed. GTP effect was dose-dependent with half-maximal enhancement of epinephrine stimulation at 1.0 μM GTP. The GTP effect was not hormone-receptor mediated, since no shift was seen of the epinephrine dose-response curve toward higher sensitivity. GTP enhancement of epinephrine stimulation occurred over a wide range of ATP concentrations (0.01–3.0 mM) and affected the substrate K_m only minimally. GTP-enhanced activity thus occurred through increased V_{max} of the hormone-sensitive adenylate cyclase.—**Katz, M. S., J. S. Partilla, M. A. Piñeyro, and R. I. Gregerman.** Essential role of GTP in epinephrine stimulation of human fat cell adenylate cyclase. *J. Lipid Res.* 1981. **22:** 113–121.

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A catecholamine-responsive adenylate cyclase has been described in human fat cells (1–4). Previous studies of human fat cell "ghosts" and purified membranes have shown marked enhancement of epinephrine-sensitive activity by the GTP analog 5′-guanylyl-imidodiphosphate (GMP-P(NH)P), but have been unable to demonstrate any consistent effect, stimulatory or inhibitory, of GTP itself (2, 3, 5). In this paper we describe a stimulatory GTP effect on the catecholamine-sensitive enzyme which is markedly temperature-dependent. This GTP effect is the first evidence for a role of naturally occurring nucleotides in the regulation of human fat cell adenylate cyclase.

MATERIALS AND METHODS

Materials

GTP (sodium salt), cAMP (sodium salt), theophylline, creatine phosphate (di-Tris salt), creatine phosphokinase, L-epinephrine bitartrate, and dithiothreitol were from Sigma. [α-^{32}P]ATP, tetra (triethylammonium) salt (10–30 Ci/mmol), and [G-^{3}H]cAMP, ammonium salt (30–50 Ci/mmol), were from New England Nuclear. [8-^{14}C]cAMP (35–50 mCi/mmol) was obtained from Schwarz-Mann. Purified sodium fluoride was from Fisher, bovine serum albumin (fraction V) from Armour, and GMP-P(NH)P from ICN. A single lot of collagenase (Worthington Biochemical, type I), which was previously found to give sufficient yield of fat cells (6), was used in all experiments.

ATP and purified ATP

For most experiments and unless otherwise designated, the best commercial grade of ATP (disodium salt) from Sigma was used. However, for certain experiments ATP free of guanine nucleotide impurities was prepared by chromatography of the commercial material. ATP was purified by anion exchange chromatography by a preparative scale adaptation of the

Abbreviations: GMP-P(NH)P, 5′-guanylyl-imidodiphosphate.

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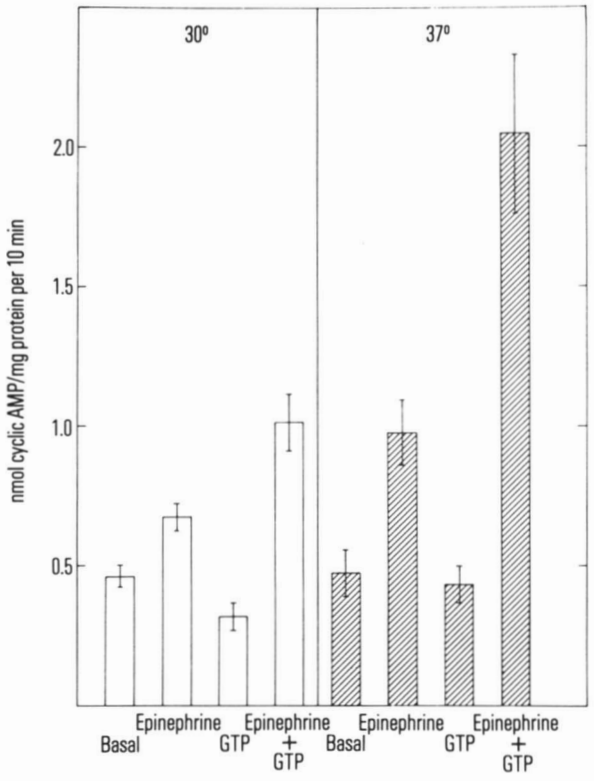

Fig. 1. GTP-enhancement of epinephrine-sensitive adenylate cyclase in human fat cell ghosts: effect of incubation temperature. Bars represent mean activity ± S.E. from 18 experiments at 30 or 37°C under standard assay conditions as described in Materials and Methods. Concentration of epinephrine, 100 μM; GTP, 100 μM. At 30 and 37°C Epinephrine > Basal and Epinephrine + GTP > Epinephrine, $P < 0.001$; GTP < Basal, $P < 0.001$, at 30° but not at 37° (P values from paired Student's t test). Activity at 37°C > 30°C for Epinephrine ($P < 0.02$), Epinephrine + GTP ($P < 0.001$), by unpaired Student's t test. NaF (5 mM)-stimulated activities (not shown): 3.45 ± 0.45 at 30°, n = 12; 7.82 ± 1.57 at 37°, n = 8.

method of Hurlbert et al. (7). This procedure is simpler than DEAE chromatography (8) and produces ATP completely free of GDP and GTP.[2]

Source of tissue

Samples of subcutaneous fat (5–15 g) were obtained from the anterior abdominal wall of adult (more than 18 years old) male and female patients undergoing elective abdominal surgery. All subjects gave informed consent for the procedure and were given a general anesthetic after an overnight fast. Patients with diabetes mellitus or poor nutritional status were excluded. Individual experiments used fat from a single patient.

For experiments with rat fat, epididymal fat pads from 2-month-old and 12-month-old male Wistar rats were used. These animals were from an outbred strain raised at the Gerontology Research Center. When young animals were used, epididymal fat pads from two rats were pooled for each experiment.

Preparation of fat cell membranes ("ghosts")

Fat samples taken at the beginning of surgery or at killing of the animals were placed immediately in normal saline warmed to 37°C. Isolated fat cells were prepared by collagenase digestion as described by Rodbell (9). Fat cell ghosts were prepared by the method of Birnbaumer, Pohl, and Rodbell (10) with the exception that 1 mM dithiothreitol was included in the lysing and suspending media. Ghosts were assayed for adenylate cyclase activity within 15 min after preparation.

Adenylate cyclase assay

Enzyme activity was measured by the method of Salomon, Londos, and Rodbell (11) in a volume of 50 μl. The standard assay mixture contained 1.5 × 10^6 dpm [α-^{32}P]ATP; 1.0 mM ATP; 25 mM Tris·HCl, pH 8.2; 5.0 mM $MgCl_2$; 2 mM cAMP; 10^4 dpm [^{3}H]cAMP; 0.1% albumin; 10 mM theophylline; and an ATP-regenerating solution consisting of 11 mM creatine phosphate and 1 mg/ml creatine phosphokinase. Ten minute-incubations were carried out in duplicate at 37°C (unless otherwise specified) in a shaking water bath. Reactions were initiated by addition of 20 μl of suspended membrane ghosts (5–20 μg protein) and were terminated by addition of 100 μl of a "stopping" solution (pH 7.6, 34 mM sodium lauryl sulfate, 40 mM ATP, 12 mM cAMP), followed by heating for 5 min in a boiling water bath. Approximately 10^4 dpm [^{14}C]cAMP was added prior to heating to monitor recovery during column isolation of cAMP. Recoveries of [^{3}H] and [^{14}C]cAMP in any single tube were essentially identical, indicating that no destruction of cAMP by phosphodiesterase occurred during incubations.

Protein determination was by the method of Lowry et al. (12). Triple labeled calculations were facilitated by use of a Fortran computer program written in our laboratory and performed in a Raytheon Computer Model 706.

RESULTS

GTP enhancement of epinephrine-sensitive adenylate cyclase: effect of temperature

Fig. 1 shows the effects of GTP on epinephrine-sensitive adenylate cyclase activity in human fat cell

[2] Katz, M. S., et al. Unpublished results.

ghosts at incubation temperatures of 30 and 37°C for observations made at a single point in time (10 min). The limitation of this manner of examining the data is discussed below (see Time courses). At both temperatures, epinephrine (100 μM) alone *appeared* to stimulate enzyme activity, and GTP (100 μM) enhanced stimulation by epinephrine. Stimulatory effects of hormone and nucleotide were greater at 37°C than at 30°C. At 37°C basal activity did not appear to be affected by GTP. Epinephrine stimulated adenylate cyclase 2.3-fold relative to basal activity while GTP doubled epinephrine-stimulated activity to 4.8-fold. At 30°C, basal activity was the same as at 37°C. However, at 30°C GTP inhibited basal activity. This inhibitory effect at 30°C made comparisons of epinephrine and GTP effects as "fold increases" (activity relative to basal) misleading. In these terms, however, epinephrine-stimulation was slight (1.6-fold), though statistically significant, and was increased by GTP only to 2.4-fold.

Other comparisons of activities at the two temperatures as fold-increases could also be made, but for the same reason were also misleading. Thus, activity with epinephrine plus GTP versus GTP alone was 4.3-fold at 30°C, not much less than the 5.1-fold effect at 37°C, and the difference between these was not statistically significant. Nevertheless, Fig. 1 does show clearly that, expressed in absolute terms, epinephrine-stimulated activity and enhancement by GTP were substantially greater at 37°C than at 30°C. Moreover, the effect of GTP on basal is really not the important issue. As shown below, the meaningful comparison is between epinephrine alone and epinephrine plus GTP.

Time courses

Fig. 2 shows time courses of adenylate cyclase activities in the absence and presence of GTP and epinephrine at incubation temperatures of 30 and 37°C. At both temperatures, initial basal activity fell within 2–4 min to a lower rate which was then linear up to 10 min. GTP added alone caused early inhibition at both temperatures. Inhibition by GTP was constant at 30°C, while at 37°C inhibition decreased with time, such that by 10 min enzyme activity in the presence of GTP equaled basal activity seen in the absence of the nucleotide (Figs. 1 and 2). Higher temperature had been found by others to shorten initial GMP-P(NH)P inhibition of basal rat fat cell adenylate cyclase activity (13).

The most striking result revealed by the time course studies was that the effect of epinephrine added alone was to sustain the initial high rate of basal activity rather than to produce a true stimulation of basal activity. Thus, at both temperatures the "stimulation" produced by epinephrine was more apparent than

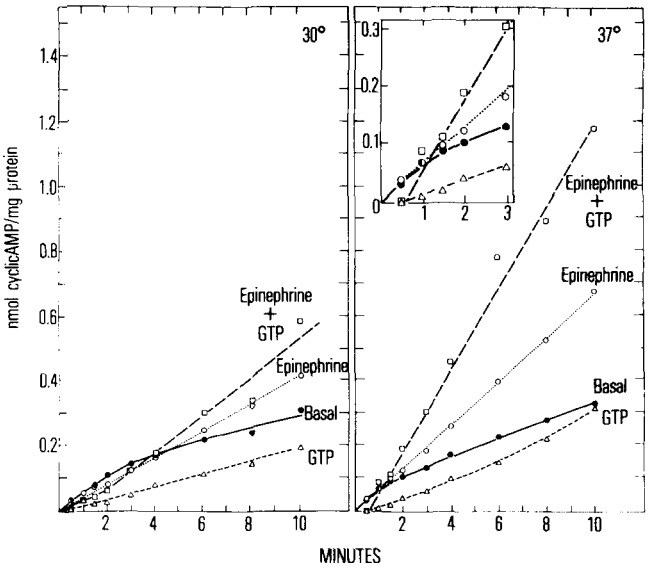

Fig. 2. Time courses of human fat cell adenylate cyclase activities. Fat cell ghosts were incubated at 30 or 37°C under standard assay conditions. Concentration of epinephrine, 100 μM; GTP, 100 μM. Individual points are means from two experiments; each experiment measured the time courses at both temperatures in ghosts from different patients.

real, the result of fall-off of basal (unstimulated) activity and the maintenance by epinephrine of the initial basal rate. However, when GTP was added along with epinephrine, a reaction velocity was achieved which clearly exceeded that of hormone added alone. This occurred despite an initial GTP-induced inhibitory lag which was longer at 30°C than at 37°C (3 min versus 30 sec, respectively). By 10 min, GTP clearly enhanced the epinephrine effect at both temperatures. Clearly, any simple assessment of the magnitude of hormone or nucleotide effects based on a single point in time can be grossly misleading.

Dose-response curves for epinephrine plus GTP

Epinephrine was titrated in the presence of several concentrations of GTP to determine whether increased epinephrine-sensitive activity in the presence of GTP might be due to displacement of the dose-response curve for epinephrine. **Fig. 3** shows that the threshold for epinephrine stimulation (1.0 μM) was unchanged by the presence of concentrations of GTP up to 100 μM. The concentration of epinephrine required for half-maximal stimulation (K_a = 3.4–4.7 μM epinephrine) was also unaffected by GTP at any concentration. Although not apparent in Fig. 3, maximum response to epinephrine alone was at 100 μM hormone (see Fig. 6). The enhancement of this response by GTP (Figs. 1 and 2) obviously cannot be accounted for by a shift in epinephrine dose-response.

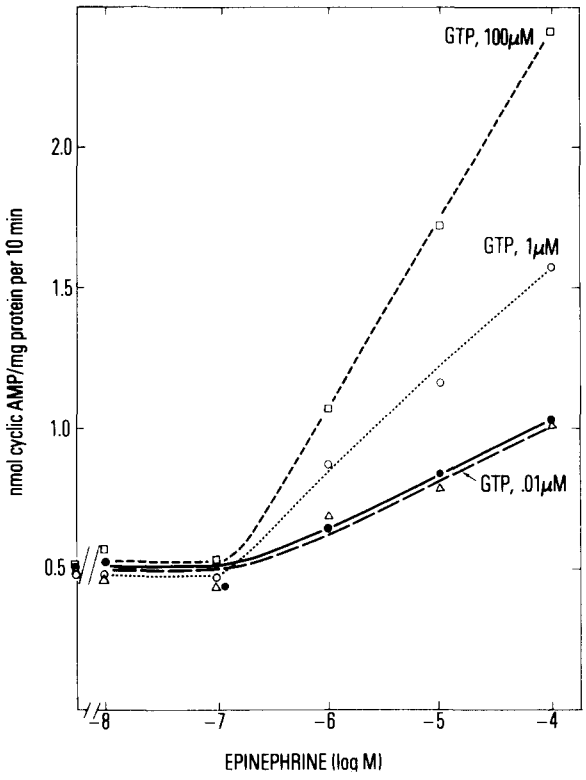

Fig. 3. GTP enhancement of epinephrine-sensitive adenylate cyclase activities. Fat cell ghosts were incubated at 37°C under standard assay conditions. Individual points are mean values from five experiments each measuring (in ghosts from a different patient) the epinephrine dose–response in the absence and presence of GTP at 0.01 μM, 1.0 μM, and 100 μM.

Dose-response curves for GTP

Fig. 3 shows that enhancement of epinephrine stimulation by GTP was dependent on the concentration of GTP as well as that of epinephrine. **Fig. 4** shows that maximum GTP enhancement of epinephrine-stimulated activity occurred at 100 μM GTP for maximally effective (100 μM) and suboptimal (1.0 μM) concentrations of epinephrine. Half-maximal GTP effect was at 0.8 μM GTP. Interestingly, the magnitude of the GTP effect was also dependent on epinephrine concentration, with the greatest enhancement by GTP occurring when stimulation by hormone alone was maximal (i.e., at 100 μM epinephrine). GMP-P(NH)P enhancement of epinephrine-stimulated human fat cell adenylate cyclase has been found by us (data not shown) and others (4) to show similar dose-response to that of GTP in Fig. 4.

Effects of ATP concentration and kinetic characteristics

The effect of GTP on epinephrine-stimulated adenylate cyclase was examined at ATP concentrations ranging from 0.01 mM to 3.0 mM (**Fig. 5**). GTP (100 μM) reproduced its apparent doubling of the epinephrine response at ATP concentrations below 3 mM. In constast to these results at 37°C, we previously observed less epinephrine effect at 30°C (with and without GTP) over this range of ATP concentrations and no response at all below 0.5 mM ATP (3).

In order to assess further the effects of epinephrine and GTP on the human fat cell enzyme, kinetic data from Fig. 5 were fitted to Eadie-Hofstee plots of V versus V/S. In the presence of excess Mg^{2+} (5 mM), ATP at concentrations up to 1 mM were assumed to exist as the active Mg·ATP substrate complex; enzyme activities at 3 mM ATP were excluded from kinetic analysis. Values of K_m for basal and basal plus GTP are only approximations because of the deviations from linearity of their time-courses. However, the mean K_m value calculated for basal enzyme was 55 μM, which was similar to that previously reported for human fat cell adenylate cyclase (2). K_m of the basal enzyme in the presence of GTP was 84 μM. The results with GTP appear to differ from those in a rat fat cell adenylate cyclase system where GTP (added as a 1:1 GTP:Mg^{2+} mixture) raised the K_m in both the presence and absence of epinephrine (14). In our system, the substrate K_m in the presence of epinephrine alone was 111 μM and was decreased slightly by GTP to 92 μM ($P < .05$). Since the calculated K_m values were all no more than one-tenth of the 1 mM ATP used in our

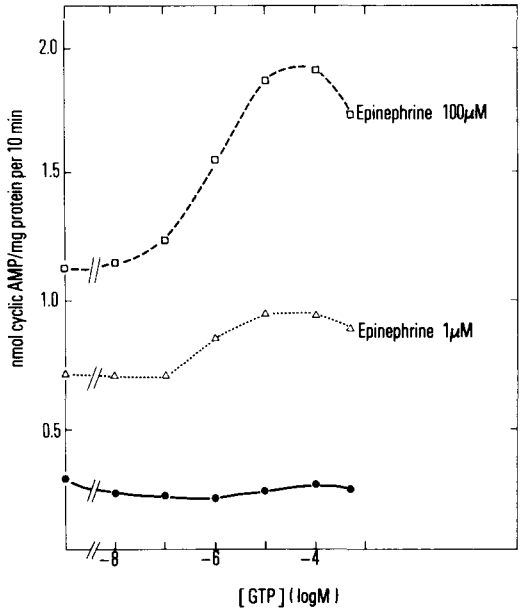

Fig. 4. GTP enhancement of epinephrine-sensitive adenylate cyclase in human fat cell ghosts: GTP dose–response. Ghosts were incubated at 37°C under standard assay conditions. Values are means of duplicate determinations from a single experiment. GTP had essentially no effect at any concentration in the absence of epinephrine.

routine incubations, the enhancement of epinephrine stimulation by GTP could not be accounted for by the slight reduction of K_m we observed in the presence of GTP.

Is GTP an absolute requirement for an epinephrine effect?

Kimura, Nakane, and Nagata (15), have recently shown that commercially available ATP contains as much as 0.2% contamination with GTP. Using ATP from which GTP had been removed, they demonstrated an absolute requirement for GTP by glucagon-sensitive adenylate cyclase in rat liver plasma membranes (8). These observations presumably explain the requirement for GTP by a number of hormone-sensitive adenylate cyclase systems under assay conditions of low but not high ATP content (16–21). The question for our study was not whether GTP was an absolute requirement for hormone stimulation, which seemed apparent from the data presented above, but whether epinephrine alone, in the absence of GTP, could even maintain the initial rate of basal activity (Fig. 2). If the ATP in our standard assay system were contaminated with as much as 0.2% GTP (15), 2 μM GTP (0.2% × 1 mM ATP = 2 μM GTP impurity) might have been present in our assay, an amount in the range of GTP enhancement of epinephrine-stimulated activity (c.f. Fig. 4).

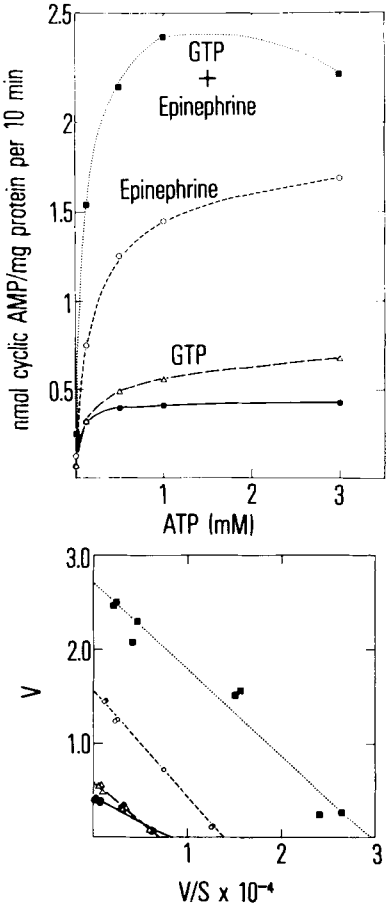

Fig. 5. Effects of ATP concentration on human fat cell adenylate cyclase in the absence and presence of epinephrine, 100 μM; GTP, 100 μM; and epinephrine + GTP. Fat cell ghosts were incubated at 37°C with a range of ATP concentrations under otherwise standard assay conditions of Mg^{2+} excess (5 mM). Values are means of duplicate determinations from a single, replicable experiment. Data were plotted by the method of Eadie-Hofstee (V versus V/S). Linear regression and resultant slopes (slope by Eadie-Hofstee plot = $-K_m$) were calculated. K_m values were compared and P values determined (see text) by the Student's t test using standard errors of slopes.

TABLE 1. Effect of ATP substrate purity on human fat cell adenylate cyclase response to epinephrine

Addition	Adenylate Cyclase Activity	
	A	B
	nmol cyclic AMP/mg protein per 10 min	
None	0.12	0.11
Epinephrine 1.0 μM	0.22	0.17
Epinephrine 100 μM	0.28	0.24

Aliquots of a fat cell ghost preparation were incubated at 37°C under standard assay conditions (1 mM ATP) using either A) Commercially available ATP or B) ATP from which GTP impurity was removed. Removal of GTP (and GDP) from ATP by ion exchange chromatography is described in Materials and Methods.

Using purified ATP from which GTP and GDP contaminants had been removed by ion exchange chromatography (see Materials and Methods), it was apparent that suboptimal (1.0 μM) and optimal (100 μM) concentrations of epinephrine were "stimulatory" even when this purified ATP was used as substrate (**Table 1**).[3] Presumably, therefore, initial basal activity was maintained by epinephrine even in the absence of added GTP or GDP. We cannot, of course, rule out the presence in fat cell ghosts of sufficient endogenous membrane-bound guanine nucleotides to have exerted some regulatory influence on adenylate cyclase in our assay system.

Comparison of GTP and GMP-P(NH)P effects on epinephrine-stimulated adenylate cyclase

In the human fat cell adenylate cyclase system, GTP and GMP-P(NH)P presumably act as in other tissues at the same nucleotide regulatory site (13). Excess GTP has previously been shown to block the stimulatory action of GMP-P(NH)P on the human fat cell

[3] Removal of GMP impurity was apparently not necessary because only a minimal amount of GTP is produced from GMP in the presence of 1 mM ATP and a regenerating system (22).

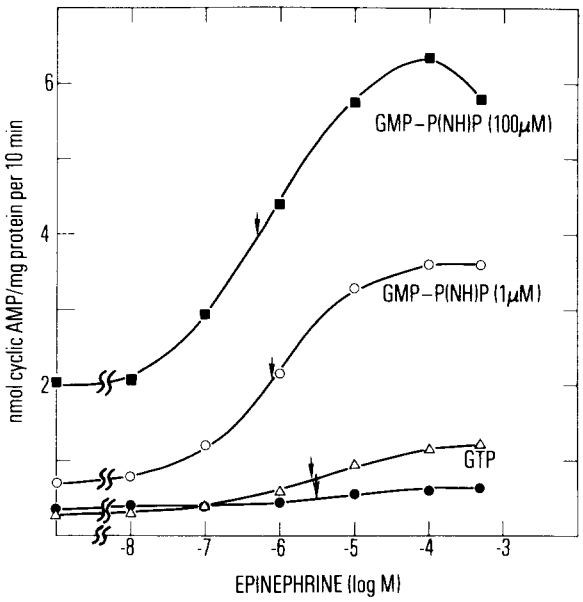

Fig. 6. GTP versus GMP-P(NH)P effects on epinephrine-stimulated adenylate cyclase. Human fat cell ghosts were incubated at 37°C under standard assay conditions. Individual points are means from two experiments, each measuring (in ghosts from a different patient) epinephrine dose–response in the absence and presence of GTP (100 μM) or GMP-P(NH)P (1.0 μM, 100 μM). Arrows show epinephrine concentration giving half-maximal stimulation (K_a).

enzyme (4). Furthermore, both nucleotides produce early inhibition of enzyme activity which is diminished by time, elevated temperature, or hormone (Fig. 2; refs. 3, 4).

In our system, GTP and GMP-P(NH)P both enhanced epinephrine-stimulated enzyme activity in a nucleotide dose-dependent manner, with the same threshold for enhancement (0.1–1.0 μM) and the same concentration required for maximum enhancement (100 μM, see Fig. 4; data for GMP-P(NH)P not shown). **Fig. 6** compares the effects of GTP and GMP-P(NH)P on the dose-response of epinephrine-stimulated activity. GMP-P(NH)P, even at a suboptimal concentration (1.0 μM), clearly had much greater effect than the optimal concentration of GTP (100 μM). As shown above, GTP had no effect on the concentration of epinephrine producing half-maximal stimulation (K_a from Fig. 6, 2.8–3.2 μM epinephrine). In contrast, GMP-P(NH)P appeared to shift the epinephrine dose-response toward slightly higher sensitivity, decreasing the K_a for epinephrine to 0.5–0.8 μM.

GTP and GMP-P(NH)P also differed in the extent to which their effects on enzyme activity were reversible after preincubation with fat cell ghosts followed by extensive washing. **Table 2** compares the effects of the two nucleotides on ghosts which were either *A*) assayed immediately with epinephrine and nucleotide, or *B*) preincubated with epinephrine and nucleotide, washed thoroughly, and then assayed. Stimulation by GTP and epinephrine was abolished in the preincubated-washed preparations, while stimulation by GMP-P(NH)P and epinephrine withstood repeated washing. GMP-P(NH)P alone stimulated enzyme activity even under conditions of preincubation and washing (data not shown). Previous work found irreversible activation by GMP-P(NH)P plus catecholamine in frog erythrocyte membranes (23) and by GMP-P(NH)P alone in rat (24) and human (4) fat cell systems. In our system, persistent stimulation by GMP-P(NH)P but not by GTP probably reflects GMP-P(NH)P resistance to membrane GTPase, which is thought to deactivate adenylate cyclase by hydrolyzing GTP from the active enzyme-GTP complex (25).

GTP effect on epinephrine-sensitive adenylate cyclase in rat fat cell ghosts

The epinephrine-sensitive adenylate cyclase of rat fat cell ghosts was also examined for responsiveness to GTP at 30 and 37°C. In 10-min incubations, GTP (100 μM) enhanced epinephrine (100 μM) stimulation by 20% at 30°C and 36% at 37°C ($P < 0.02$ and <0.001, respectively). Time courses at 37°C with ghosts from both young and adult animals showed linear epinephrine-stimulated activity up to 10 min and basal activity which fell off after about 5 min (data not shown). The time courses in the rat ghosts, unlike those of human material, did not show any suggestion of mere preservation by epinephrine of an initial rate of basal

TABLE 2. Adenylate cyclase activities of human fat cell ghosts preincubated with guanine nucleotides and epinephrine and then washed

Addition	Adenylate Cyclase Activity	
	A (Control)	B (Preincubated-washed)
	nmol cyclic AMP/mg protein per 10 min	
None	0.4	0.3
Epinephrine + GTP	2.0	0.3
Epinephrine + GMP-P(NH)P	8.9	10.2

Aliquots of fat cell ghosts from a single patient were either A (Control): resuspended and incubated at 37°C under standard assay conditions, or B (Preincubated-washed): resuspended in a stabilizing solution (1 mM ATP, 10 mM DTT, 11 mM creatine phosphate, 1 mg/ml creatine phosphokinase, 5 mM $MgCl_2$, 0.1% BSA, 1 mM $KHCO_3$, 25 mM Tris·HCl, pH 7.4) and preincubated at 25°C for 10 min. Preincubated ghosts were washed four times with stabilizing solution, then resuspended and incubated at 37°C under standard assay conditions. Epinephrine, GTP, and GMP-P(NH)P were each added at a concentration of 100 μM to the assay system for A or to the preincubated membranes for B. A (Control) activities were as expected for standard assay conditions (c.f. Fig. 6). Ghosts preincubated without additions and subsequently assayed in the presence of epinephrine plus GTP showed activity of 1.4 nmol cyclic AMP/mg protein per 10 min.

activity. Rather, epinephrine added to rat fat cell ghosts produced an immediate and linear response which was slightly enhanced by GTP.

DISCUSSION

GTP enhances epinephrine-sensitive adenylate cyclase activity in human fat cell ghosts (Fig. 1). Previous studies have shown enhancement of hormone activity by GMP-P(NH)P (3, 4), but we present here the first evidence that the naturally occurring nucleotide is involved in the stimulation of the epinephrine-sensitive enzyme of this system. Figs. 1 and 2 show that enhancement of epinephrine stimulation by GTP is temperature-dependent, with much greater effect at 37 than at 30°C. This temperature dependence probably explains the minimal or absent enhancement of epinephrine stimulation by GTP previously reported (3, 5), since those experiments were performed at 30°C.

The time course studies at 30 and 37°C (Fig. 2) clarified the characteristics of epinephrine stimulation and the effect of GTP. Epinephrine alone seemed merely to preserve the initial rate of basal activity. At both temperatures in the absence of hormone, the initial rate of reaction of basal activity fell off rapidly. In a sense, therefore, the "stimulation" of activity produced by hormone alone is an artifact of comparing a linear rate in the presence of hormone with a falling rate of basal activity. Only in the presence of both GTP and hormone is a new, higher activity state achieved.

Enhancement of epinephrine stimulation by GTP did not involve changes of K_m for substrate or K_a for hormone (Figs. 3, 5, and Results). Similar dose–response curves for epinephrine in the presence and absence of GTP (Figs. 3 and 6) suggest that the nucleotide did not change the affinity of membrane receptors for hormone. In contrast to these effects of GTP, increased catecholamine sensitivity in the presence of GMP-P(NH)P was found in human fat cell ghosts by us and others (Fig. 6; ref. 4), in rat fat cell ghosts (21), and in frog and turkey erythrocyte membranes (23, 26). The lack of effect of GTP on epinephrine dose–response in fat cell ghosts also differs from the observation in rat liver membranes of a GTP-induced shift of glucagon dose–response to greater sensitivity (27).

In contrast to our results in human fat cell ghosts, no effect of GTP on hormone-sensitive enzyme at 37°C was found in a plasma membrane fraction from human fat cells (2). Since those purified membranes would presumably have contained even less GTP than our ghosts, it is unlikely that this aspect of the purity of the membrane preparations accounts for our different results. On the other hand, the earlier work (2) did not concern itself with the possible contamination of the ATP substrate with GTP. Furthermore, enhancement of activity by GTP in ghosts, as opposed to more highly purified membranes, could conceivably reflect retention in the ghosts of cytosol components that regulate enzyme activity. Soluble factors which are stimulatory for epinephrine-sensitive adenylate cyclase have been described in cytosol from rat fat, liver, and heart (28–31). Other explanations for the difference between purified membranes (2) and our ghosts might relate to alterations of membranes during their isolation, as has been reported for liver (32), or, conceivably, to our use of subcutaneous as opposed to omental fat (2). Recent work has indicated regional differences in the characteristics of human fat cell adenylate cyclase (33).

It was of interest to compare our results using human fat cell ghosts with those from rats. With the rat preparations, GTP also showed statistically significant enhancement of epinephrine stimulation at both 30 and 37°C, but the effect was small. Unlike the results with human fat cell ghosts, however, even in the absence of added GTP epinephrine produced clear stimulation of the rat fat cell adenylate cyclase, rather than merely preservation of a high initial rate of basal activity. These differences between the time courses of the human and rat preparations extend to others that have been previously described (3). Since age influences hormonal responsiveness of adenylate cyclase activity of rat fat cell ghosts (34), we examined the possibility that an age rather than a species difference might account for the contrasting time course results between the human and rat materials. However, experiments comparing 2-month-old (young) and 12-month-old (adult) rats showed that at both ages epinephrine produced an immediate and linear stimulation that was increased slightly by GTP (data not shown). We conclude, therefore, that the contrasting time course characteristics of the human and rat fat cell adenylate cyclases of ghosts are species related.

The present results indicate that epinephrine stimulation of human fat cell adenylate cyclase requires GTP, but such a requirement has not, in fact, been shown for the enzyme from the rat. Recent work by Cooper et al. (35) clearly showed that β adrenergic-stimulation of adenylate cyclase in rat fat cell membranes can occur independent of added GTP. Their study of GTP effects did not present time courses of enzyme activities in the absence and presence of hormone and GTP. However, assuming linearity with time in their experiments, isoproterenol-stimulation of adenylate cyclase of membranes did *not* require addition of GTP, even though added nucleotide clearly

enhanced hormone-stimulated activity. These authors also showed that stimulation of rat fat cell adenylate cyclase by another hormone, namely ACTH, did require GTP under conditions where stimulation by isoproterenol was nucleotide-independent (35). Additionally, a previous study of rat fat cell membranes demonstrated that, without added guanine nucleotide, ACTH did not produce an increased rate of adenylate cyclase activity but rather merely sustained an initial high basal rate that subsequently fell in the absence of hormone; only in the presence of a guanine nucleotide (GMP-P(NH)P) was a higher rate of activity achieved by hormone (13). This situation with ACTH in the rat is similar to the effect of epinephrine which we have shown in human fat.

Our observations on human fat cell adenylate cyclase show another difference from previously observed GTP effects on the enzyme from the rat. Work with rat fat cell membranes (35, 36) demonstrated that increasing concentrations of GTP caused progressive increase of catecholamine-sensitive activity up to a peak activity, but that further increases of GTP concentration resulted in a decline (i.e., inhibition) from peak activity. Moreover, this bimodal stimulation and inhibition of hormone-stimulated activity by GTP was temperature-dependent, such that elevating the assay temperature shifted the biphasic GTP dose-response curve to the right (35). In contrast, in our human fat cell ghosts at 37°C, the GTP effect on epinephrine-sensitive activity was stimulatory at concentrations of GTP from 0.1 μM to 0.1 mM, with no inhibitory phase of the GTP dose–response (Fig. 4). Slight decline from peak stimulatory effect of GTP did occur at 0.5 mM GTP (Fig. 4), but that high concentration may have been enough guanine nucleotide to compete for Mg^{2+} with the 1 mM ATP in our assay and thereby diminish the concentration of active ATP·Mg substrate. Additionally, no substantial inhibitory portion of the GTP dose–response curve was found in human fat cell ghosts at 30°C (Ref. 4, Fig. 5) although at this temperature the magnitude of the stimulatory phase of the GTP dose–response was much less than at 37°C. Inhibition by GTP of hormone-sensitive adenylate cyclase activity found by others in the rat (35, 36) but not by us in the human may represent a species-related difference of nucleotide effect, but may also relate to the use of substantially different assay conditions as well as different enzyme preparations (ghosts versus membranes).

On the basis of past studies of GTP effects on a number of adenylate cyclases (16–21) and the direct demonstration of a requirement for GTP by glucagon-sensitive adenylate cyclase in rat liver plasma membranes (8), it is widely believed that hormone-stimulation of mammalian adenylate cyclase is absolutely dependent on GTP. Our results with human fat cell ghosts would agree with this view, but results by others using human (2) and rat fat cell membranes (35) have so far failed to demonstrate an absolute guanine nucleotide requirement for hormone stimulation in this system. Overt nucleotide dependence, however, as opposed to a modulator effect, may depend on the state of the enzyme and its membrane environment.

The information we present here is essential for optimal quantitation of adenylate cyclase(s) in conjunction with studies on the regulation of lipolysis in human fat cells. We are currently investigating further the conditions required for maximum stimulatory effect of GTP on adenylate cyclase(s) of human fat cell preparations. Such conditions may facilitate study of the regulatory function of the naturally occurring nucleotide that has previously not been possible at suboptimal conditions of assay.

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